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(54) Title: BREAD IMPROVER COMPRISING BILE SALT AND PHOSPHOLIPASE A

(57) Abstract: The present invention relates to a bakery product improver composition comprising a bile salt and phospholipase A.

BREAD IMPROVER COMPRISING BILE SALT AND PHOSPHOLIPASE A

The present invention relates to a bakery product improver composition and to a method for improving the quality of bakery products by adding an improver composition.

Improver compositions for bakery products, e.g. bread improvers, are complex mixtures which may contain various functional ingredients such as oxidizing and reducing agents (e.g. ascorbic acid, L-cysteine), enzymes (e.g.alpha-amylase, hemicellulase), emulsifiers in the form of dough conditioners (e.g. DATEM (diacetyl tartaric acid ester of mono/diglycerides) and SSL (sodium stearoyl lactylate)), or crumb softeners (monoglycerides like GMS (glycerol mono stearate)), and carriers or bulk materials (starch, sugars, etc.). Many of the commonly used bread improvers contain one or more emulsifiers added to improve loaf volume and/or crumb softness and/or crust crispyness. From the consumer's point of view it is advantageous to diminish the use of emulsifiers because they are chemical additives.

It is well-known that the quality of a baked product, defined in terms of the product's volume, its internal structure and crumb softness and the various organoleptic parameters depends on the rheological properties of the dough, in particular dough elasticity and extensibility. These properties are dependent on the quality of the wheat flour used and more specifically on the quality of the gluten proteins. The gluten network developed from these proteins during mixing and proofing determines the elasticity and extensibility of the dough.

Emulsifiers interact with the insoluble components of flour such as gluten proteins. When this occurs the emulsifier acts at the liquid–solid interface. Most dough conditioners are anionic emulsifiers (Sahi, S.S. (1994) in Food Technology International Europe, pp 60–62, Sterling Publ. Lim., London). The mechanism of action of these materials appears to be related to the ability of the lipophilic tails of the emulsifier to bind to the protein hydrophobic patches introducing negative charges into the complex. The introduction of the negative charge neutralizes some of the positive charge, thus reducing the overall charge of the protein-emulsifier complex. The reduction in electrostatic repulsion promotes protein

aggregation in the dough and hence has a strengthening effect (Green, F.C., Kasarda, D.A. (1971) Cereal Chem. 48, 601-606).

Besides the influence on dough rheology the gluten network is also strongly involved in the ability of the dough to retain gas. In addition to gluten also the hemicellulose and lipid fractions of the flour play an important role in this phenomenon. Optimisation of the hemicellulose fraction is commonly done by application of enzymes like cellulase, hemicellulase, pentosanase or (endo)xylanase.

Besides hemicellulose, wheat flour contains approximately 2.2-2.9% lipids. The flour lipids can be divided into starch lipids (0.8-0.9%) and non-starch lipids (1.4-2.0%). Whereas the starch lipids consist mainly of polar lysophospholipids, the non-starch lipids consist of about 40% neutral triglycerides and 40% polar phospho- and glycolipids. Around 30% of these phospholipids consist of lysophospholipids (A-C. Eliasson and K. Larsson (1993) in Cereals in Breadmaking, Marcel Dekker Inc., New York, USA, pp 32-33). As stated by K. Larsson (1983) in Lipids in Cereal Technology (ed. P.J. Barnes, Academic Press, New York, USA) in particular *polar* lipids contribute to good baking properties. L. Acker and G. Becker (1972) Gordian 72, 275-278 have already shown that the more polar *lyso*phospholipids perform superior to the corresponding phospholipids in breadmaking. Optimisation of the wheat flour lipids fraction may be done by addition of commercially available soya lecithins or enzyme-modified soya lecithins. In the latter case the *lyso*phospholipid fraction is enriched by enzymatic modification of the phospholipids present. For this conversion often Lecitase® (NOVO Nordisk, Denmark), being phospholipase A2, is used.

For optimisation of the wheat flour lipids fraction it is also possible to hydrolyse the phospholipids *in situ* in the dough by adding the enzyme phospholipase A as part of a bread improver. E.g. EP-A-109244 and WO98/26057 describe this use of phospholipase A in breadmaking. In these patents the effects of addition of phospholipase A in the absence and presence of added soya lecithin on bread characteristics are described. In all cases a positive effect on loaf volume and crumb characteristics is seen, however, most explicitly in the case of the combination of enzyme and exogenous substrate. In EP-A-109244 pancreatic phospholipase A2 is applied to improve loaf volume. In

WO 98/26057 the phospholipase A activity preferably is a phospholipase A_1 activity.

Phospholipase A1 is able to hydrolyse phospholipids as e.g. present in wheat flour, soya or egg lecithin, preferentially in the presence of Triton X-100 (US 5,538,874). Phospholipase A2 is able to catalyze the hydrolysis of molecularly dispersed substrates, however, becomes superactivated at certain organised lipid/water interfaces (e.g. H. Brockerhoff & R.G. Jensen (1974) Lipolytic Enzymes, Academic Press, New York). A commonly used assay for phospholipase A2 activity (W. Nieuwenhuizen et al. (1974) Meth. Enzymol. 32B, 147-154) mixes egg-yolk lipid and sodium deoxycholate (NaDC) to produce a mixed micellar structure.

EP-A-0914777 discloses a dough improving composition composed of a protein complexed with a saponin or a bile salt. US 4,115,739 describes emulsifier compositions containing bile salts which can be used in bread-making. US 3,833,738 describes the preparation and use of either the soluble fraction or the insoluble fractions (celloflour) of alfalfa or clover after being treated with an aqueous basic solution and simultaneously or serially digestion with pancreatin and a bile containing material. The alfalfa or clover extracts are added to food stuffs in view of their nutritional characteristics.

Figure 1: Effect of anPLA in the presence of NaTDC on the loaf volume of Dutch tin bread baked using the short process. The loaf volume of the reference was 4,359 ± 80 mL.

Figure 2. Effect of anPLA in the absence and presence of NaDC on the loaf volume of pao frances bread baked using a standard proof time.

Figure 3. Same as Figure 2, but with an additional 1 hour proof time

Surprisingly it has been found that adding phospholipase A to a dough in combination with very small quantities of bile salts results into baked products having characteristics at least equivalent to those found for baked products to which doughs commercially available emulsifiers have been added. In these cases no exogenous lipids in the form of either soya or egg-yolk lipids, or enzyme modified lipids need to be added. Moreover, the number of phospholipase A units/kg of flour added to obtain the maximum baking result can

be reduced when compared to the effect of adding the combination of phospholipase A and exogenous lipids. The present invention therefore provides a bread improving composition which comprises a bile salt, more preferably sodium taurodeoxycholate (NaTDC), sodium taurocholate (NaTC), sodium deoxycholate (NaDC) and/or sodium cholate (NaC) and phospholipase A. Besides phospholipase A and bile salt the improver composition may also comprise oxidants (e.g. ascorbic acid), reducing agents (e.g. L-cysteine), oxidoreductases (e.g. glucose oxidase), polysaccharides modifying enzymes (e.g. alpha-amylase, hemicellulase, branching enzymes, etc.), and/or protein modifying enzymes (endoprotease, exoprotease, branching enzymes, etc.). By improver composition is meant a composition which has a favourable effect on dough and/or bread or other bakery products and which is added to the basic elements of preparing a dough. The basic elements are water, flour, yeast and optionally salt.

Composition and dosage of the improver ingredients should be advantageously chosen so that in the prepared dough is added phospholipase A1 in between 10 and 50,000 PLU/kg flour, preferably in between 20 and 25,000 PLU/kg flour and more preferably in between 50 and 10,000 PLU/kg flour, or phospholipase A2 in between 100 and 20,000 U/kg, preferably in between 250 and 10,000 U/kg and more preferably in between 500 and 5,000 U/kg and a bile salt or a combination of bile salts in between 0.005 and 0.5 % (w/w, calculated on the flour), preferably in between 0.01 and 0.1%, and more preferably in between 0.02 and 0.08%.

One unit (PLU) of phospholipase A1 is defined as the amount of enzyme producing 1 micromole of free fatty acid per minute under the conditions of the test. The release of free fatty acids from lecithin in a buffer comprising 2 % alpha-lecithin (Calbiochem, >94% L-alpha-phosphatidylcholine), 1 % Triton X-100, 20 mM citrate, pH 4.0, incubated for 10 minutes at 40°C. The reaction is stopped by placing the incubation tubes in a boiling water bath for 10 minutes. The free fatty acid level is determined by titration with 0.01 M KOH to pH 9.0.

One unit (EYU) of phospholipase A2 is defined as the amount of enzyme producing 1 micromole of free fatty acid per minute in the egg-yolk test under the conditions described by Nieuwenhuizen et al. (1974) Meth. Enzymol. 32B, 147-154.

For both phospholipase A1 and phospholipase A2 all commercially available products may be used. The phospholipase A may be of mammalian, snake or bee venom, and microbial origin and may be obtained via r-DNA technology. A suitable commercial source is Lecitase® 10-L (NOVO Industri A/S, Denmark) a phospholipase A2 preparation extracted from pig pancreas.

The present invention will be further demonstrated by the following examples. It should be noted that the present invention is by no means limited to these examples. All Fermipan® and Fermizyme® products are from DSM Bakery Ingredients, Delft, The Netherlands.

Reference example

Baking performance of bile salts as such

The baking performance of bile salts as such was tested in pup loaves. Preparation of pup loaves in a standard baking process was done by mixing 200 g of wheat flour (5–10° C), 1.4 g Fermipan® instant yeast, 3.2 g salt, 0.8 g $CaCl_2 .2H_2O$, 25 ppm ascorbic acid, 10 ppm fungal alpha-amylase Fermizyme® P200, 60 ppm of fungal hemicellulase Fermizyme® HS2000 and 112 mL water (30-35°C) in a pin mixer for 6 minutes and 15 seconds. The dough temperature is 27°C. Machineability of the dough is analysed by hand by the baker. Directly after mixing the dough is divided into two pieces of 150 g, rounded and proofed for 45 min. in a proofing cabinet at 30°C. Afterwards the doughs are moulded and given an intermediate proof of 25 min. at 30°C. At the end of this period the doughs are shaped and panned and given a final proof of 70 min. at 30°C. Afterwards the fully proofed doughs are baked in an electric oven at 230°C for 20 min.. After cooling down to room temperature the volumes of the loaves are determined by the rape seed displacement method. Afterwards break and shred (the external of the breads) is analysed. After 24 hrs of storage in a closed box at room temperature the crumb quality is assessed by the baker. As bile salts sodium deoxycholate (Aldrich Chem. Co., ≥98% pure) and sodium taurodeoxycholate (Aldrich Chem. Co., ≥95% pure) were applied. As a reference DATEM (Panodan ®185, Grindsted Products Inc., Braband, Denmark) was used. Results of DATEM and of both biosurfactants separately and in (equimolar) combination are gathered in Table 1.

Table 1

NaTDC (% w/w)	NaDC (% w/w)	DATEM (% w/w)	Machineability after mixing	Bread & shred *	Crumb * structure	Loaf volume (mL)
0	0	0	Good	6	7	528
0.028	0	0	Good / little stiff	6.5	5.5	542
0.056	0	0	Good / little stiff	6	6	562
0.112	0	0	Good / little more stiff	7.5	7.5	605
0.22	0	0	Good / little more stiff	7.5	8	595
0	0.011	0	Good / little stiff	6.5	6.5	525
0	0.022	0	Good / little stiff	6	6.5	535
0	0.045	0	Good / stiff	6	6	532
0	0.09	0	Good / stiff	6.5	6	536
0.014	0.011	0	Good / little stiff	6.5	6	544
0.028	0.022	0	Good / little stiff	7	6	545
0.056	0.045	0	Good / little stiff	7.5	6	551
0	0	0.037	Good	6	7	552
0	0	0.075	Good / little stiff	6.5	7	553
0	0	0.15	Good / little stiff	7	7.5	565
0	0	0.3	Very good / little stiff	8.5	7.5	593
0	0	0.5	Very good / little stiff	8.5	7	602

* The scale is in between 0 and 10, whereby 10 is the best score

From these results it may be concluded that NaTDC is showing a baking performance very similar to DATEM, both in dough and bread characteristics. Albeit the level of dosage of NaTDC is only about 20% of the level at which DATEM is applied. Applying NaDC results are not significantly different from the reference. Combination of equimolar quantities of NaTDC and NaDC show some improvement when compared to the reference, however, not large when compared to the results obtained with NaTDC alone.

Example 2

Baking performance of combination of NaTDC and phospholipase A

The same breadmaking method was used as described in Example 1. Lecitase®10L was used as phospholipase A (PLA). In Table 2 the results are gathered of baking trials in which phospholipase A alone and in combination with various levels of dosage of NaTDC were tested. PLA applied in this recipe did not show very much effect on loaf volume and other bread characteristics. However, surprisingly combinations of PLA and NaTDC gave very significant results. Combining only 0.056% (w/w) NaTDC and only 1000 EYU/kg PLA resulted into a baking result equivalent to that of 0.5% (w/w) DATEM. As an alternative it is also possible to add 0.11% (w/w) NaTDC and only 500 EYU/kg. At the level of 0.11% (w/w) NaTDC a saturation effect is seen. Addition of PLA does not really contribute to the increase of the loaf volume already obtained by adding the bile salt alone (results in Table 1). In those cases PLA may also be overdosed.

From these results it may be concluded that PLA and the bile salt NaTDC are synergistic in their effects on bread characteristics: in combining both ingredients a smaller amount of both is needed to obtain at least the same result as being obtained when only one of both ingredients is added. An advantage of the combination is that during the process the doughs containing the combination of PLA and NaTDC becomes less stiff than those containing DATEM. At the same time they fully perform in dough conditioning.

Table 2

PLA/NaTDC synergism			Machineability after mixing	Break & shred	Crumb structure	Loaf volume (mL)
DATEM (%)	NaTDC (%)	PLA (EYU/kg)				
-	-	-	Good	6	7	542
0.3	-	-	Good	7.5	8	590
0.5	-	-	Very good / little stiff	8	8	621
-	-	2500	Good / little stiff	5	6	556
-	-	7500	Good / very stiff	6.5	6	539
-	0.028	-	Good / little stiff	6.5	5.5	542
-	0.028	2500	Good / stiff	7	8.5	583
-	0.028	5000	Good / very stiff	7.5	8	594
-	0.056	-	Good/ little stiff	6	6	562
-	0.056	250	Good / little stiff	7.5	7.5	595
-	0.056	375	Good / little more stiff	7.5	8	592
-	0.056	500	Good / little more stiff	7.5	7.5	606
-	0.056	1000	Good / stiff	8	7.5	615
-	0.056	2500	Good / stiff	8	8.5	610
-	0.056	5000	Good / stiff	6.5	8.5	588
-	0.056	7500	Good / stiff	7	7.5	585
-	0.112	-	Good / little more stiff	7.5	7.5	605
-	0.112	500	Good / stiff	8	8	629
-	0.112	2500	Good / stiff	8	8.5	612
-	0.112	5000	Good / stiff	7	8.5	597
-	0.224	-	Good / little more stiff	7.5	8	595
-	0.224	2500	Good / stiff	7	8	601

Example 3

Baking performance of combination of NaDC and phospholipase A

As shown in Example 1 sodium deoxycholate (NaDC) when applied in preparation of pup loaves did not show very much improvement of loaf volume. Combinations of PLA (Lecitase® 10L) and NaDC were applied to produce pup loaves according to recipe and method described in Example 1. Results are summarised in Table 3. From this Table it may be learned that combination of a very low level of addition of NaDC of 0.022% (w/w) and 2,500–5,000 EYU/kg PLA results into a volume comparable to that of addition of 0.3–0.5% (w/w) DATEM. Both lower (≤250 EYU/kg) and higher (≥7,500 EYU/kg) levels of PLA cause less increase of loaf volume.

Addition of NaDC levels ≥0.04 % (w/w) in combination with levels of PLA in between 1,250 and 7,500 U/kg all result in more or less the same loaf volumes. Introducing NaDC results into more stiffness of the dough directly after mixing. In combination with PLA stiffness is even more intensively present. Later on in the process these effects disappear. At 0.022% (w/w) NaDC and 2,500–5,000 EYU/kg PLA break & shred and crumb quality are equivalent to the quality obtained with 0.3% DATEM. A similar quality is seen when 0.045% (w/w) NaDC plus 2,500 EYU/kg PLA are added.

Table 3

PLA/NaDC synergism			Machineability after mixing	Break & shred	Crumb structure	Loaf volume (mL)
DATEM (%)	NaDC (%)	PLA (EYU/kg)				
-	-	-	Good	6	7	522
0.3	-	-	Good	7.5	8	590
0.5	-	-	Very good / little stiff	8	8	621
-	0.011	-	Good / little stiff	6.5	6.5	525
-	0.011	250	Good / little more stiff	6.5	6	533
-	0.011	500	Good / little more stiff	6.5	7	543
-	0.011	1250	Good / little more stiff	6.5	6	545
-	0.011	2500	Good / stiff	6.5	6	547
-	0.011	5000	Good / stiff	7	8	555
-	0.011	7500	Good / stiff	8	7.5	575
-	0.022	-	Good / little stiff	6	6.5	535
-	0.022	250	Good / little stiff	6.5	6.5	550
-	0.022	500	Good / little more stiff	7	7	546
-	0.022	1250	Good / little more stiff	8	7.5	576
-	0.022	2500	Good / little more stiff	7.5	7.5	607
-	0.022	5000	Good / stiff	8	8.5	622
-	0.022	7500	Good / very stiff	7.5	8	581
-	0.045	-	Good / stiff	6	6	532
-	0.045	2500	Good / very stiff	8	8	613
-	0.045	5000	Good / very stiff	7.5	8.5	614
-	0.045	7500	Good / very stiff	7.5	8	609
-	0.09	-	Good / stiff	6.5	6	536
-	0.09	2500	Good / very stiff	7.5	8.5	612
-	0.09	5000	Good / very stiff	8	8.5	614
-	0.09	7500	Good / very stiff	8	8.5	613

From these results it may be concluded that PLA and NaDC are very synergistic in their effects on the various dough and bread characteristics. Both are not very effective when applied alone, together they show a very distinct positive effect both on loaf volume and on other bread parameters.

Example 4

Baking performance of combination equimolar levels of NaTDC & NaDC and phospholipase A

In Example 1 it was shown that the combination of equimolar parts of sodium taurodeoxycholate (NaTDC) and sodium deoxycholate (NaDC) did not result into an impressive increase of loaf volume. This combination is here combined with Lecitase® 10L in preparing pup loaves via recipe and bread making method as described in Example 1. In Table 4 the results are summarised. From these results it is clear that a similar relationship in volume versus dosage level is found as seen for the combination of PLA and NaDC. At 0.05% (w/w) (being the combination of 0.028% NaTDC and 0.022% NaDC) and 500 EYU/kg PLA dosage level a bread volume is obtained similar to that of 0.5% (w/w) DATEM. At higher bile salt levels lower loaf volumes are found. Table 4 teaches that the external of the bread improves when the combination of bile salts is applied. Crumb structure is not further improved. When the combination of bile salts is applied in combination with PLA besides loaf volume, break and shred also the crumb structure is strongly improved.

From the results gathered in this Table it is also clear that when the bile salt mix is exchanged by small quantities of one of the commercial emulsifiers a similar effect on bread characteristics is found. From these results it is clear that the quantity of emulsifier added to the dough recipe may be strongly reduced by addition of phospholipase A. An extra advantage of using the combination of phospholipase A and either bile salt or commercial emulsifier is that the crumb structure is much more regular and fine than obtained when only emulsifier is obtained. In all these cases machineability was good. In some cases the dough is somewhat more stiff, however, this small effect did absolutely not influence dough handling during moulding and shaping.

Table 4

PLA/NaTDC + NaDC synergism (equimolar quantities of both detergents)			Machineability after mixing	Break & shred	Crumb structure	Loaf volume (mL)
DATEM (%)	Bile salts (%)	PLA (EYU/kg)				
-	-	-	Good	6	7	522
0.3	-	-	Good	7.5	8	590
-	0.025	-	Good / little stiff	6.5	6	544
-	0.025	500	Good / little stiff	6.5	8	544
-	0.05	-	Good / little stiff	7	6	545
-	0.05	500	Good / little stiff	8	7.5	626
-	0.10	-	Good / little stiff	7.5	6	551
-	0.10	500	Good / little stiff	8	8	597
0.075	-	2500	Little slack	8	7.5	596
SSL (%)						
0.075	-	2500	Good / little stiff	8	8	607

Example 5

Baking performance of combination of NaTDC and phospholipase A produced by r-DNA technology

Phospholipase A was produced by cloning the porcine pancreas gene encoding for prophospholipase A2 and bringing it to expression in *Aspergillus niger*. The activated and purified enzyme (anPLA; "an" stands for Aspergillus niger) showed to have a specific activity of 1,050 EYU/mg. The baking performance in combination with bile salt was tested in the production of Dutch tin bread. Preparation of tin bread in a standard baking process was done by mixing 3500 g wheat flour (80% Kolibri and 20% Ibis (Meneba, Holland)) (about 21°C), 77 g compressed yeast (Konings®), 70 g salt, 25 ppm ascorbic acid, 10 ppm fungal alpha-amylase Fermizyme® P200, 15 ppm fungal hemicellulase Fermizyme® HSP6000, 2030 mL water (8–15°C) and either 0.3% DATEM (Panodan 80 CP; Grindsted Products Inc., Braband, Denmark) or various quantities of anPLA as such or in combination with 0.03% sodium taurodeoxycholate (NaTDC) in a spiral mixer (Hobart) for 2 minutes at speed 1 and for about 6 minutes at speed 2 to put in 125 Wh. of energy. The dough

temperature was 28°C. Machineability of the dough was analysed by hand by a qualified baker. Directly after mixing, the dough was divided into 6 pieces of 875 g, rounded and proofed for 35 minutes in a proofing cabinet at 34°C and 85% RH. At the end of this period the doughs were punched, shaped and panned and given a final proof of 75 minutes in a proofing cabinet at 38°C and 87% RH. Afterwards the fully proofed doughs were baked in an electric oven at 210°C for 30 minutes. After cooling down to room temperature the volumes of the loaves of bread were determined by the rape seed displacement method. After 16–24 hours of storage in sealed polyethylene bags at room temperature the crumb quality was assessed by a qualified baker.

Machineability of all doughs directly after mixing was good. Doughs including higher levels of anPLA became a little stiff, while corresponding doughs also containing NaTDC became more stiff. Also doughs containing 0.3% DATEM were a little stiff directly after mixing. Effects of various ingredients on loaf volume are shown in Figure 1. From these results it is clear that the enzyme anPLA as such does only contribute to loaf volume when at least 5000 EYU/kg flour are added. In combination with 0.03% NaTDC 2500 EYU/kg flour of anPLA already gives a loaf volume being about 4% higher than found when 0.3 % DATEM was applied. Break and shred (the external of the breads) was very nice for both DATEM breads and breads containing the combination of anPLA and NaTDC. Crumb texture of DATEM containing breads was regular but a little open, while crumb texture of breads containing the combination of anPLA and NaTDC was regular and fine. Overviewing these results it may be concluded that the combination of anPLA and NaTDC results in improved dough and bread characteristics, which scores are even better than those found for applying 0.3% DATEM.

Example 6

Baking performance of combination of anPLA and soy lecithin in the absence and presence of NaDC

The baking performance of anPLA in combination with bile salt was also tested in production of pao frances, a bread roll currently produced in South-West Europe and South America. Preparation of pao frances in a standard baking

process was done by mixing 3500 g wheat flour (Margriet; Meneba, Holland), 57% water (8–15°C), 0.9 % Fermipan® instant dried yeast, 2% salt, 2% enzyme active soya flour, 0.5% sugar, 80 ppm ascorbic acid, 15 ppm fungal alpha-amylase Fermizyme® P200, 8 ppm fungal hemicellulase Fermizyme® HSP6000 and optionally 0.25% DATEM (Panodan 80 CP) or 0.3% soy lecithin in combination with various quantities of anPLA in the absence and presence of sodium deoxycholate (NaDC) in a Diosna spiral mixer for 2 minutes at speed 1 and for about 6 minutes at speed 2 to put in 100 Wh. of energy. Dough temperature directly after mixing was 28°C. A dough piece of 2100 g was weighed and divided into 30 pieces by an automatic dough divider. Afterwards the doughs were rounded and given a first proof of 15 minutes at 32°C and 85% RH. At the end of first proof the doughs were punched and shaped in a mini-moulder, put on steel racks and given a final proof of 120 minutes at 32°C and 85% RH. Afterwards the doughs were baked in an electric oven at 225°C for 20 minutes. After cooling down to room temperature, loaf volume was determined by measuring 10 loaves together by the rape seed displacement method.

For these trials anPLA was pre-mixed with NaDC as follows: anPLA was dissolved to an activity of 15300 EYU/ml in demineralized water. NaDC was dissolved to a concentration of 15% (w/v). Afterwards the two solutions were mixed in a volume ratio of 1:1. After gentle shaking by hand the solution was put in the freezer until it was applied in bread making.

From the baking results shown in Figure 2 it is directly clear that the DC-treated anPLA is about twice as active as the non-treated anPLA. Also higher loaf volumes could be obtained in the case anPLA / NaDC mixtures were applied. In fact, loaf volumes were seen very similar to the volumes obtained by applying 0.25% DATEM. Break and shred of NaDC/anPLA containing rolls was similar to the break and shred obtained by applying 0.25% DATEM.

Besides loaf volume also dough tolerance is a very important parameter. In general in a bakery the doughs after final proofing time are not all baked at the same moment. In such cases the oven capacity is the bottleneck. For this reason dough quality should not strongly decline in a certain period of time after end of fermentation. In this case tolerance was tested by elongation of the final

proofing time with one hour (2-3 hrs). Loaf volumes obtained are gathered in Figure 3. From this Figure can be learned that:

- in all other cases increased volumes were found. Dough tolerance was good in all cases.
- the extra effect of the NaDC / anPLA mixture has disappeared. Results for both types of anPLA are now similar.
- anPLA (+/- NaDC) resulted under these conditions in similar loaf volumes as found for application of 0.25% DATEM.

Break and shred of rolls produced with anPLA as such in combination with 0.3% soy lecithin was inferior to the break and shred of rolls produced with either 0.25% DATEM or anPLA in combination with NaDC. From these results it is clear that a) premixing with NaDC results in a twice as active enzyme, and b) application of this activated enzyme in production of pao frances does not lead to increased dough intolerance.

CLAIMS

1. A bread improver composition which comprises at least one bile salt and phospholipase A.

2. A composition according to claim 1 wherein the phospholipase A is derived from mammalian, or snakes or arthropods venom, or microbial origin.

3. A composition according to claim 2 wherein the phospholipase is obtained via r-DNA technology.

4. A composition according to claim 1 wherein the bile salt is sodium taurodeoxycholate or sodium deoxycholate.

5. A dough which comprises flour, water, yeast and optionally salt and to which is added a composition as claimed in anyone of the preceding claims.

6. A dough according to claim 5 which comprises from 10 to 50,000 PLU phospholipase A1 per kilogram of flour.

7. A dough according to claim 6 which comprises from 20 to 25,000 PLU phospholipase A1 per kilogram of flour.

8. A dough according to claim 5 which comprises from 100 to 20,000 U phospholipase A2 per kilogram of flour.

9. A dough according to claim 8 which comprises from 250 to 10,000 U phospholipase A2 per kilogram of flour.

10. A dough according to claim 9 which comprises from 500 to 5,000 U phospholipase A2 per kilogram of flour.

11. A dough according to claims 5 to 10 which comprises from 0.005 % (w/w, calculated on flour) to 0.5 % (w/w, calculated on flour) of one or more bile salts.

12. A dough according to claim 11 which comprises from 0.01 % (w/w, calculated on flour) to 0.1 % (w/w, calculated on flour) of one or more bile salts.

13. A dough according to claim 12 which comprises from 0.02 % (w/w, calculated on wheat flour) to 0.08 % (w/w, calculated on wheat flour) of one or more bile salts.

14. A process for producing a bakery product which comprises forming a dough as claimed in anyone of the claims 5 to 13 and baking the dough.

15. Use of a bile salt in a bakery improving composition or preparing a dough.

16. Use of a bile salt in combination of a phospholipase in a bakery improving composition or preparing a dough.

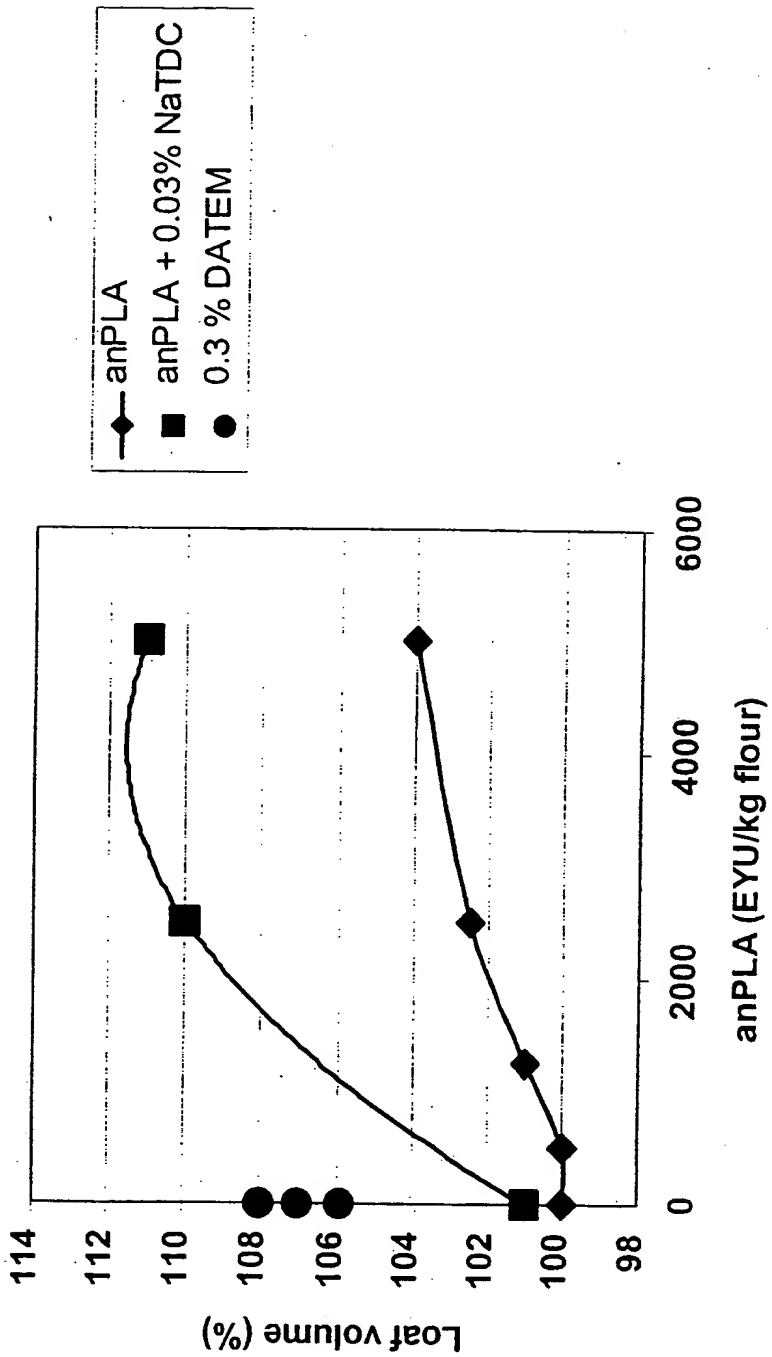
anPLA
anPLA + 0.03% NaTDC
0.3 % DATEM
Loaf volume (%)
114
112
110
108
106
104
102
100
98
0
2000
4000
6000
anPLA (EYU/kg flour)

Figure 1

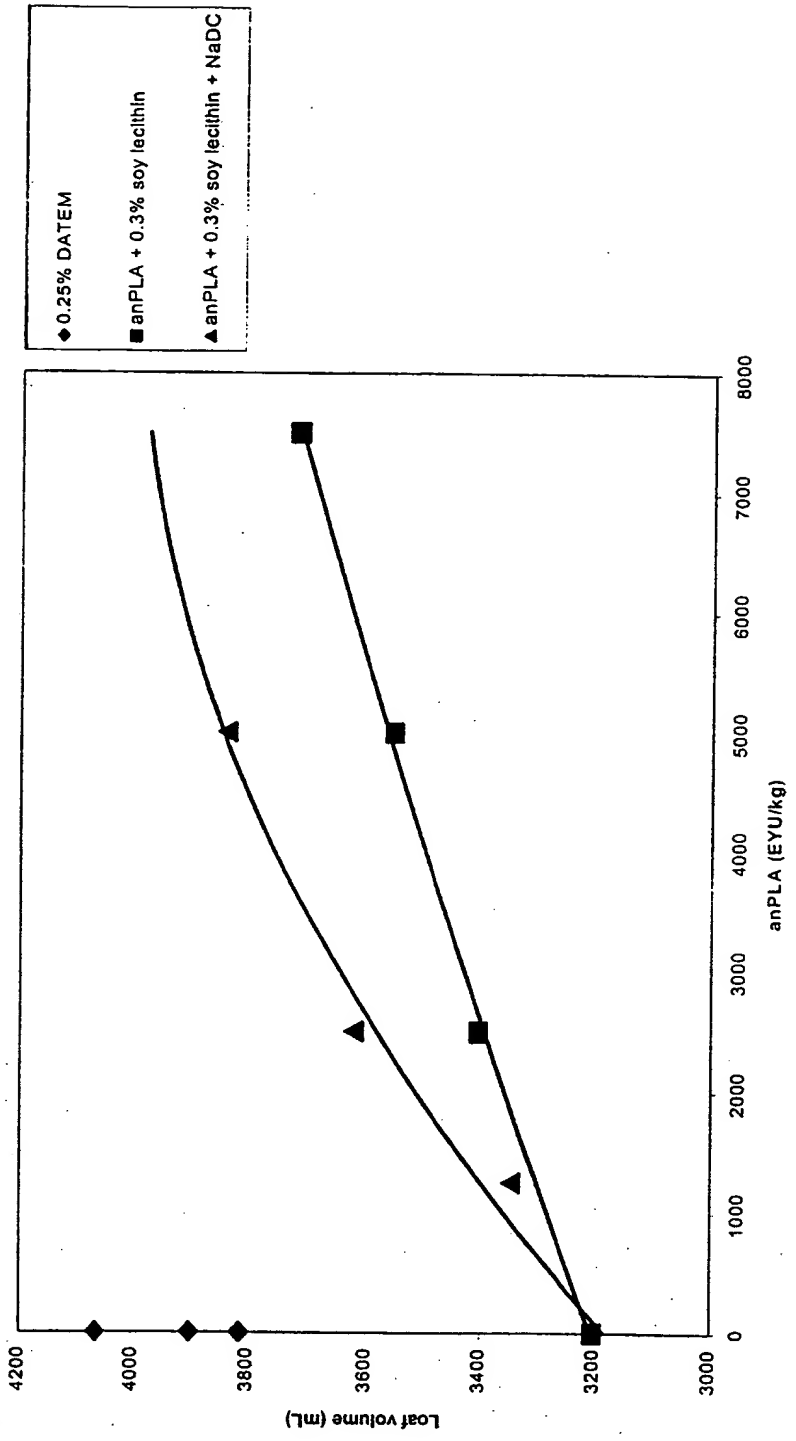
◆0.25% DATEM
■anPLA + 0.3% soy lecithin
▲anPLA + 0.3% soy lecithin + NaDC
Loaf volume (mL)
4200
4000
3800
3600
3400
3200
3000
0
1000
2000
3000
4000
5000
6000
7000
8000
anPLA (EYU/kg)

Figure 2

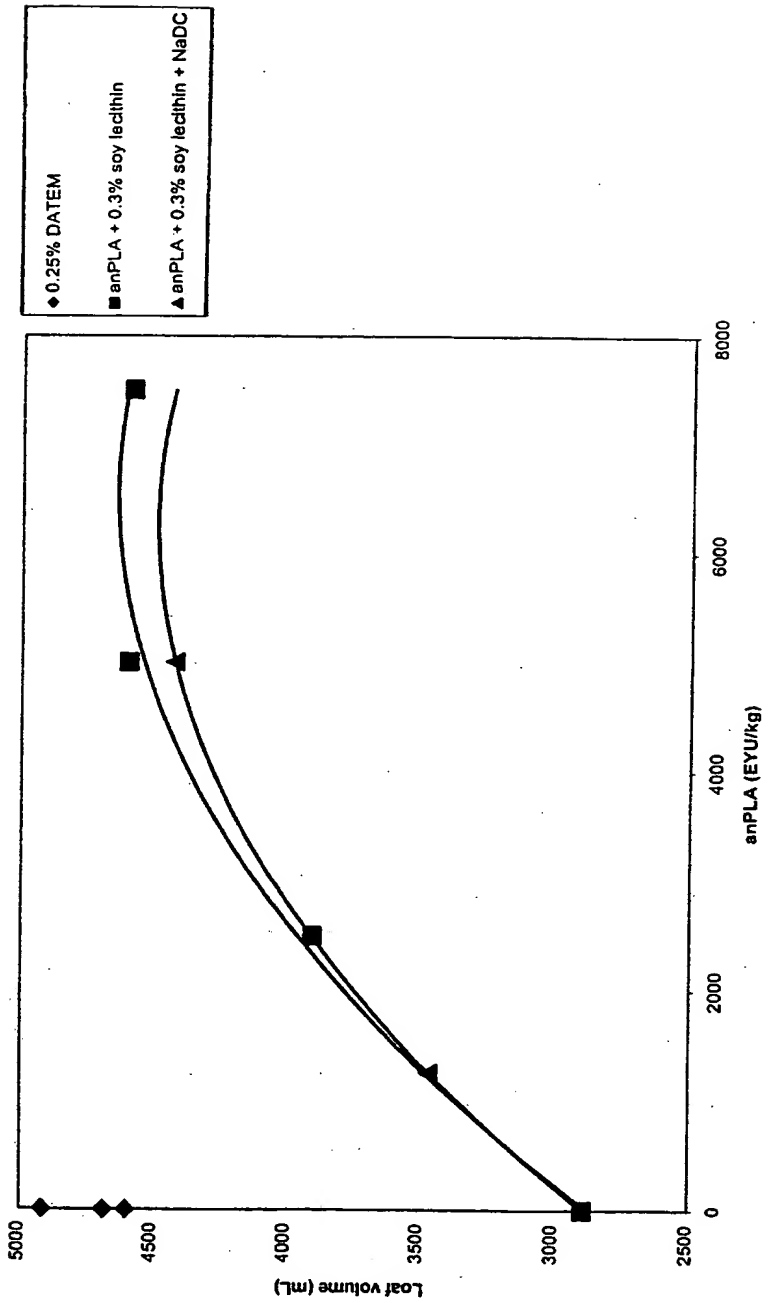
◆ 0.25% DATEM
■ anPLA + 0.3% soy lecithin
▲ anPLA + 0.3% soy lecithin + NaDC
Loaf volume (mL)
5000
4500
4000
3500
3000
2500
0
2000
4000
6000
8000
anPLA (EYU/kg)

Figure 3

INTERNATIONAL SEARCH REPORT

Intern .ial Application No
PCT/EP 00/13042

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A21D8/04 A21D2/08 A21D8/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A21D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, EPO-Internal, PAJ, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Derwent Publications Ltd., London, GB; AN 1996-382238 XP002138124 "Inhibition of starch ageing in bread and cookies" & KR 9 410 252 A (SAM LIP FOODS IND CO LTD), 22 October 1994 (1994-10-22) abstract ---	1,4,5, 14-16
X	EP 0 914 777 A (KYOWA HAKKO KOGYO KK) 12 May 1999 (1999-05-12) cited in the application page 3, line 26-41	15
A	page 5, line 25-40 examples 17,20,21,24 claims 1,3,7-10 ---	1,5, 11-14,16
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 May 2001	10/05/2001

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Intern ial Application No
PCT/EP 00/13042

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	column 2, line 7-25 column 4, line 43-55 examples II,III,IV,V	1,5,14, 16
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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

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Intern al Application No
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